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Research Article
Expression Patterns of Adrenomedullin and its Receptors in Breast Cancer Among Saudi Patients

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Abstract

Background and Objective: The adrenomedullin (AM), a peptide in the calcitonin family, acts as an angiogenic factor in tumors, with dysregulation linked to various cancers. Its receptors, AM1 and AM2, are complexes of calcitonin receptor-like receptors and RAMP2/3, but its role in breast cancer remains underexplored. This study aimed to investigate the expression levels of ADM, its receptors (RAMP2, RAMP3, CLR) and their clinical significance in breast cancer patients, to evaluate their potential as biomarkers for breast cancer diagnosis in the Saudi population. **Materials and Methods:** The 24 Saudi breast cancer patients and 24 healthy controls from King Fahad Medical City, with blood samples collected for RNA extraction and cDNA synthesis. Gene expression was measured using RT-qPCR and protein levels were assessed by Western blotting and ELISA. Statistical analysis was performed using GraphPad Prism®, with gene expression normalized to GAPDH and comparisons made using Welch's t-test. **Results:** The ADM and its receptors were significantly overexpressed in breast cancer patients as compared to control. Plasma concentrations levels of these proteins were higher in breast cancer patients with a significant difference for ADM ($p = 0.0001$) and RAMP3 ($p = 0.01$). **Conclusion:** These findings support the potential of ADM levels as biomarkers for breast cancer diagnosis in the Saudi population, indicating that blood tests for ADM could provide a less invasive alternative to biopsy procedures. Further studies with larger cohorts are necessary to validate and generalize these findings.

Key words: Calcitonin receptor, breast cancer, adrenomedullin, biomarkers, receptor activity-modifying proteins, Saudi population

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Competing Interest: The authors have declared that no competing interest exists.

Data Availability: All relevant data are within the paper and its supporting information files.

INTRODUCTION

The adrenomedullin (ADM) has a wide range of biological functions in health and disease[1]. The ADM acts as a mitogenic factor, promoting growth in several cancer types[2]. Additionally, it regulates cell proliferation, inhibits apoptosis and stimulates angiogenesis in cancer cells[3,4]. The ADM also indirectly suppresses the immune response through its binding protein, complement factor H and by modulating the expression of cytokines[5,6]. Its actions are mediated by receptor complexes formed between the Calcitonin Receptor-Like Receptor (CRLR) and a specific receptor activity-modifying protein (RAMP)[7,8].

High levels of ADM mRNA are found in the adrenal medulla, with lower expression in the heart, kidneys, lungs and certain brain regions[9,10]. The gene is also expressed in vascular endothelial and smooth muscle cells and its production is upregulated by oxidative stress, pro-inflammatory cytokines, angiotensin II and endothelin[11]. Hypoxia significantly induces ADM expression through the transactivation of its promoter by the Hypoxia-Inducible Factor 1 (HIF-1) and post-transcriptional mRNA stabilization[12]. The human ADM gene contains Hypoxia Response Elements (HREs) in its promoter. The AMBP-1, a 150 kDa protein made of 20 repetitive domains, enhances AM's receptor binding, modulates its activity and extends its half-life by protecting it from degradation[13,14]. The AM-AMBP-1 complex has shown protective effects against organ damage due to ischemia or low oxygen supply in animal models[15]. Adrenomedullin is a peptide with a ring structure and a C-terminal amide, both crucial for binding to AM receptors. Specific binding sites for AM are found in various cell types, reflecting its diverse biological functions[11]. The primary AM receptor is the calcitonin receptor-like receptor (CLR), a member of the 7-transmembrane domain G-protein-coupled receptor superfamily. The CLR requires receptor activity-modifying proteins (RAMPs) for function, with three types identified in humans: RAMP1, RAMP2 and RAMP3, each having distinct expression patterns in health and disease[16]. The CLR forms the functional AM receptor co-expressed with RAMP2 or RAMP3, while co-expression with RAMP1 results in a CGRP receptor. Both AM and CGRP receptors play critical roles in metabolism, vascular tone and inflammatory responses and are essential for normal lymphatic and blood vessel development and angiogenesis[17,18]. The adrenomedullin (ADM) dysregulation is linked to tumor progression and metastasis in various cancers, including breast cancer. Elevated ADM levels are associated with aggressive tumor characteristics, acting as an anti-apoptotic factor and promoting angiogenesis[19,20]. Most tumor cells produce ADM and express its receptors, with hypoxia enhancing ADM expression through the Hypoxia-Inducible Factor-1 (HIF-1)[21,22]. The high ADM levels correlate with lymph node metastasis in breast cancer patients, where increased vascularization is also observed[23]. The normal breast duct is composed of a layer of luminal epithelial cells and myoepithelial cells, which are supported by the extracellular matrix (ECM) and a variety of stromal cells, including endothelial and immune cells. The tumor microenvironment is critical in cancer progression, as it supplies essential nutrients and growth factors to support the metabolic needs of cancer cells[24]. Increased production of ADM by tumors and higher plasma levels of this peptide are associated with lymph node metastases, indicating its role in promoting a more aggressive metastatic phenotype[25]. In Saudi Arabia, breast cancer is the second most common cancer. Given the lack of research on ADM in the Middle East, this study aimed to investigate the relationship between ADM expression levels and pathological parameters in breast cancer, potentially establishing ADM as a biomarker for diagnostic use in the Saudi population.

MATERIALS AND METHODS

Study area: The study was carried out at the Research Center, King Fahad Medical City and King Saud University from June, 2022 to June, 2023.

Patient recruitment: The 24 Saudi breast cancer patients and 24 healthy controls, all free of chronic diseases, from King Fahad Medical City (KFMC). The study protocol was reviewed and approved by the institutional review board at KFMC (IRB number 16-258) and all participants provided informed written consent. Blood samples from 48 participants were collected following IRB guidelines and stored at -80°C until analysis. Nucleic acid isolation and cDNA synthesis.

Total RNA was extracted from freshly collected blood using the Total RNA Purification Kit with Trizol reagent (Sigma Aldrich), following the manufacturer's instructions. The RNA concentration was determined using a NanoDrop 8000 (Thermo Fisher Scientific, Epsom, UK). The cDNA synthesis was carried out with 2 µg of purified RNA using a high-capacity reverse transcription kit (Applied Biosystems, USA; Cat No. 4374967) according to the manufacturer's guidelines. The reverse transcription reaction included 10X reverse transcription buffer, 1X dNTP mix (10 mM), 1X

Table 1: Primers sequence used for the amplification of targeted genes in real-time PCR

Gene	Primer sequence (5'-3')	Temp (°C)
ADM	Forward: GTTTCCATCACCCTGATGTTATT	52.0°C
	Reverse: GTAGTTCCCTCTTCCCACGACTTAG	
RAMP2	Forward: CATCCCACTGAGGACAGCCT	60.0°C
	Reverse: GATCATGGCCAGGAGCACAT	
RAMP3	Forward: AAAGCCTTCGCTGACATGAT	50.0°C
	Reverse: CCATCTCGGTGCAGTTAGTG	
CLR	Forward: GGCAGTGGCCAATAACCAGG	56.4°C
	Reverse: ATGAGTGTCTGAGCTGATCCAGCA	
GAPDH	Forward: TTGTCAGCAATGCATCCTGC	52.0°C
	Reverse: GCTTCACCACCTTCTTGATG	

ADM: Adrenomedullin, RAMP: Receptor-like receptor and accessory proteins, CLR: Calcitonin receptor-like receptor and GAPDH: Glyceraldehyde-3-Phosphate Dehydrogenase

random primers and 15 U AMV reverse transcriptase, with a total reaction volume of 20 µL. After synthesis, the cDNA was stored at -20°C. Electrophoresis on a 1.5% agarose gel prepared with TBE buffer and 0.01% ethidium bromide confirmed the presence of the cDNA amplicon. Ten microliters of the PCR product, along with 10 µL of a DNA ladder, were loaded onto the gel and run at 110 V for 45 min, with visualization performed using the Gel Doc XR+System (Bio-Rad).

Real-time PCR: The expression levels of target genes were measured using RT-qPCR (Applied Biosystems, USA) with an SYBR® Green dye-based assay (Bio-Rad, USA). Specific primers for ADM, RAMP2, RAMP3 and CLR, designed by Primer Design, were provided in Table 1, with Glyceraldehyde-3-Phosphate Dehydrogenase (GAPDH) as the reference gene. Each reaction occurred in a total volume of 10 µL, comprising 1X Power SYBR® Green Master Mix, 0.5 µM of each primer and 2.0 µL of a 1 ng/µL cDNA sample. The experiment was performed in duplicate and relative gene expression levels normalized to GAPDH were analyzed using GraphPad Prism® software (version 8).

Western blotting: Whole blood was separated by centrifugation at 1500-2000xg for 10-15 min at room temperature, yielding three layers. The interface layer containing White Blood Cells (WBCs) was transferred to a new tube and incubated with RIPA buffer for 30 min at 4°C. Following a second centrifugation, protein extracts were denatured using 5X Laemmli loading buffer and heated at 95°C for 5 min. A total of 25 µg of protein was loaded per lane onto 10 and 15% sodium dodecyl sulfate-polyacrylamide gels, followed by electrotransfer to a Polyvinylidene Fluoride (PVDF) membrane. The membrane was blocked for 1 hr at room temperature and then incubated overnight at 4°C with primary antibodies, followed by a 1 hr incubation with HRP-conjugated secondary antibodies. After washing with PBST, the membrane was visualized using autoradiography film (Bio rad, Hyper Film, Amersham).

ELISA: The Enzyme-Linked Immunosorbent Assays (ELISAs) were conducted to measure the levels of ADM and its receptors in breast cancer and control samples. Blood samples were allowed to clot at room temperature, then centrifuged at 1,000 g for 15 min at 4°C to obtain serum, which was stored at -80°C until analysis. Soluble RAMP2, RAMP3 and CLR concentrations were assessed using specific sandwich ELISA kits (MyBioSource, USA), while soluble ADM was measured using a competitive ELISA kit (MyBioSource, USA). Results were read at 450 nm with a microplate reader and concentrations were determined against standard curves.

Statistical analysis: The statistical analysis were performed using GraphPad Prism® software (version 8). Gene expression levels for adrenomedullin and its receptors were normalized to GAPDH using the $2^{-\Delta Ct}$ method[26], where ΔCt = Ct (target gene)-Ct (GAPDH). Welch's t-test was employed to compare expression levels between breast cancer and control samples. Linear regression analysis and Spearman correlation were used to examine relationships between gene expressions, with statistical significance set at $p<0.05$ for all tests.

RESULTS

Specificity of ADM, RAMP2, RAMP3 and CLR mRNA in breast cancer: To assess the specificity of primers and the presence of these genes, the total RNA extracted was analyzed for ADM, RAMP2, RAMP3, CLR and GAPDH mRNAs using reverse transcription followed by PCR. The resulting amplicons were evaluated through agarose gel electrophoresis, as illustrated in Fig. 1, to confirm gene integrity and specificity. Each PCR product from breast cancer patients was compared with control DNA from normal human tissue. The generated fragments for ADM (Fig. 1a), RAMP2 (Fig. 1b), RAMP3 (Fig. 1c), CLR (Fig. 1d) and GAPDH measured 402, 115, 131, 98 and 187 bp, respectively. The qualitative assessment indicated successful amplification of ADM, RAMP2, RAMP3, CLR and GAPDH, serving as a positive control, while no expression was detected in the negative control (H_2O).

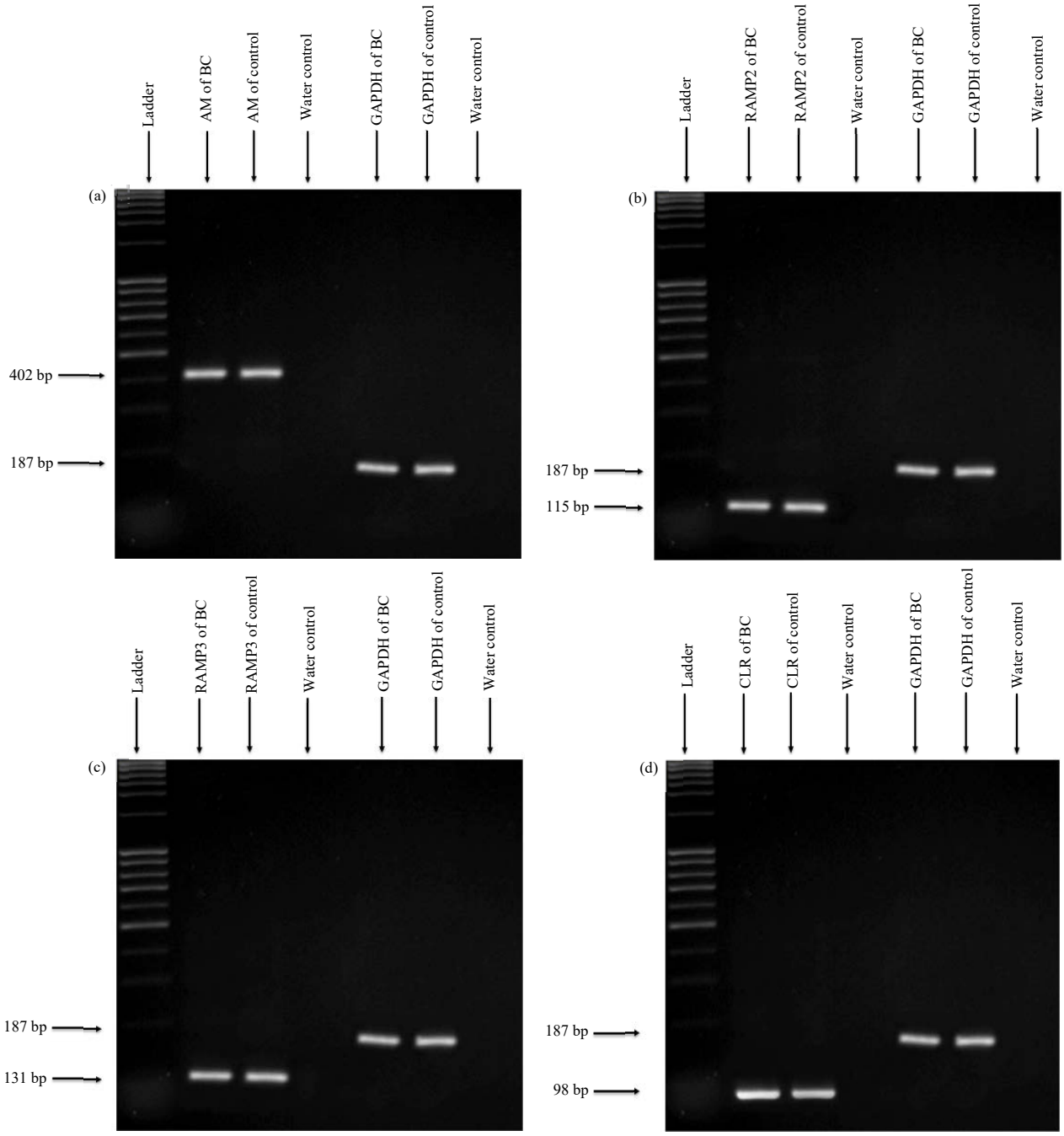

Fig. 1(a-d): Agarose gel electrophoresis for endpoint PCR to visualize the presence of expressions in breast cancer, control samples and water control, (a) ADM, (b) RAMP2, (c) RAMP3 and (d) CLR

Expression levels of ADM, RAMP2, RAMP3 and CLR mRNA in breast cancer: To further investigate ADM, RAMP2, RAMP3 and CLR expression levels in breast cancer versus normal samples, we employed RT-PCR, as shown in Fig. 2. The data were expressed as the ratio of the mRNA levels of these genes to GAPDH, a housekeeping gene. Our findings demonstrated significant differences ($p = 0.0001$) in gene expression between breast cancer and control samples. Specifically, Fig. 2 shows that the relative mRNA levels of ADM in Fig. 2a, RAMP2 in Fig. 2b, RAMP3 in Fig. 2c and CLR in Fig. 2d were notably higher in breast cancer patients compared to normal. The statistical analysis indicated that ADM levels were approximately 4 times greater in breast cancer patients, while RAMP2 and RAMP3 levels were about twice as high. The CLR showed the smallest increase among the receptors, with a slight elevation compared to normal.

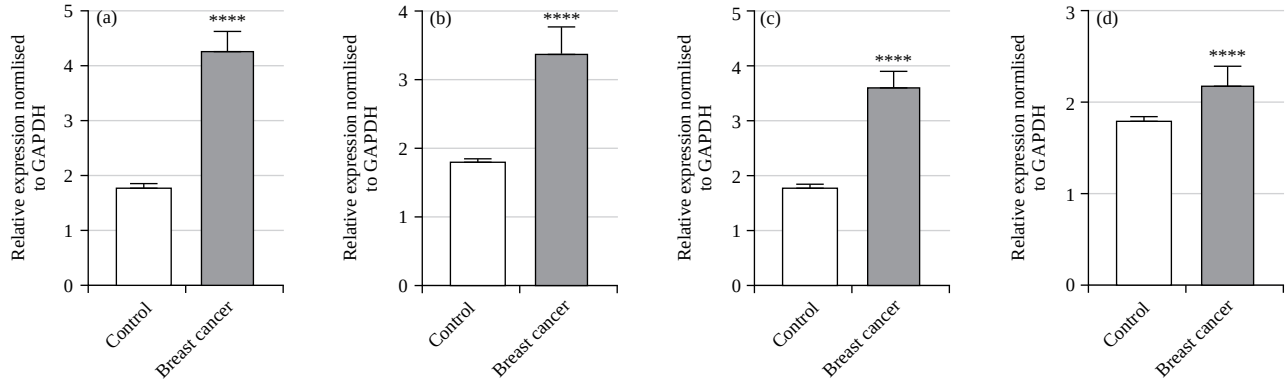

Fig. 2(a-d): Relative expressions in breast cancer and control samples normalized to GAPDH

Q-PCR used represents a statistically significant increased expression in BC patients compared to control, ****p-value = 0.0001, (a) ADM, (b) RAMP2, (c) RAMP3 and (d) CLR

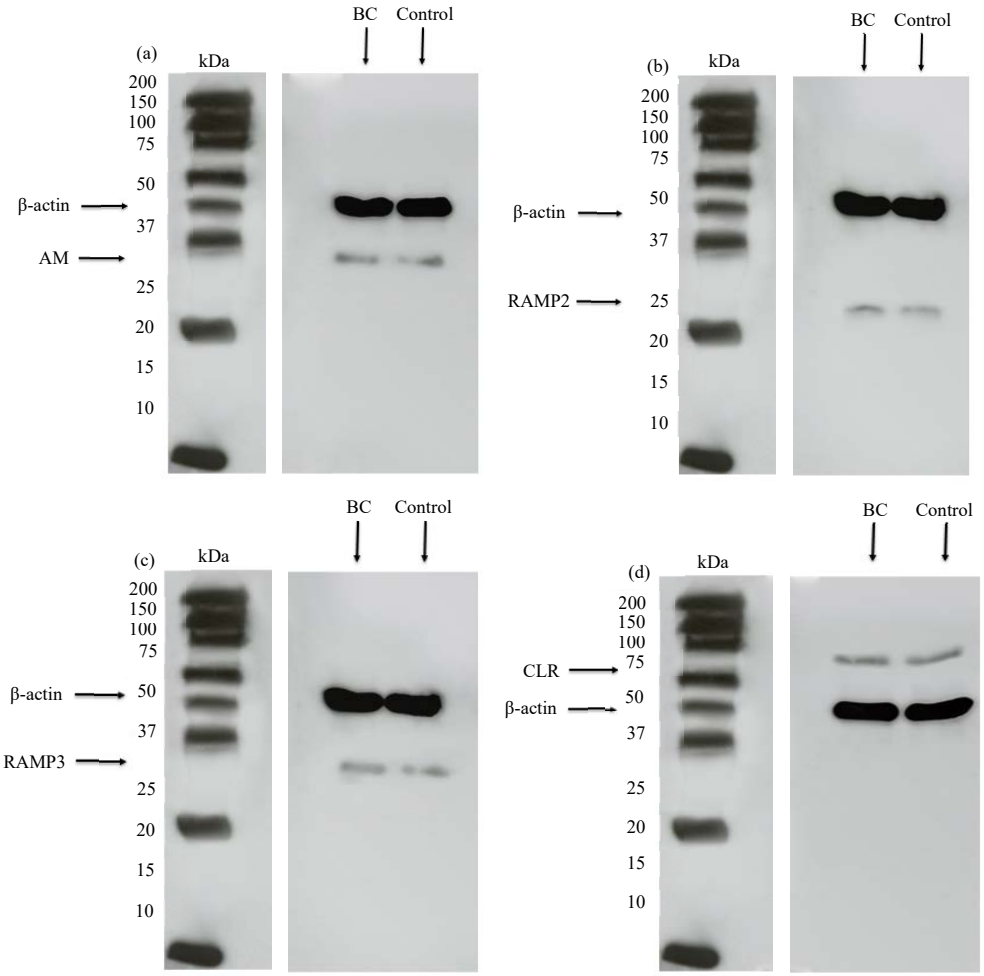

Fig. 3(a-d): Immunoblotting of AM and its receptors on protein lysates from breast cancer patients, (a) Quantification of AM expression level, (b) Quantification of RAMP2 expression level, (c) Quantification of RAMP3 expression level and (d) Quantification of CLR expression level

Expression of AM, RAMP2, RAMP3 and CLR in 15% SDS-PAGE. A band of the size 45 kDa corresponded to β-actin which shows the loading was equal in all samples, while the other band represents the proteins based on its size 28, 25, 35 and 75 kDa of AM, RAMP2, RAMP3 and CLR, respectively

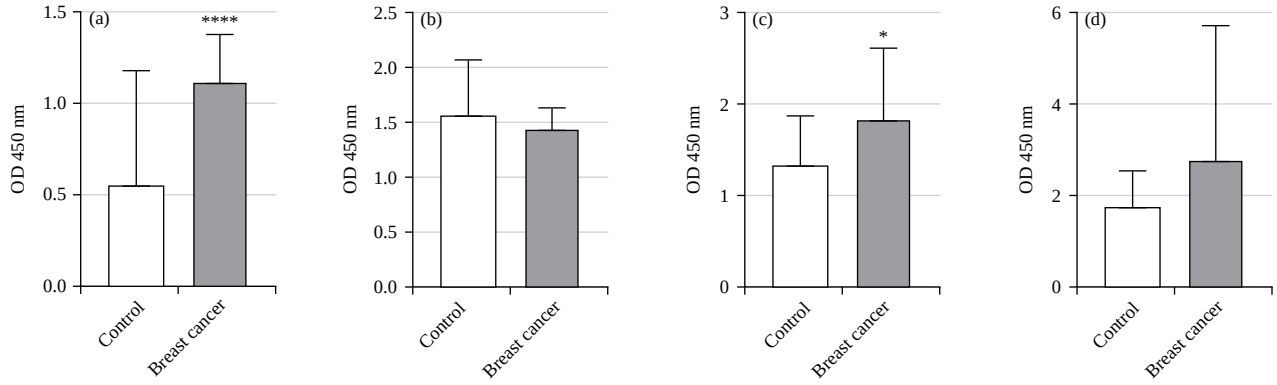

Fig. 4(a-d): Protein expression of ADM, RAMP2, RAMP3 and CLR in breast cancer virus control at the protein level by using ELISA, (a) Protein expressions of ADM, (b) Protein expressions of RAMP2, (c) Protein expressions of RAMP3 and (d) Protein expressions of CLR

(a) Protein expression of ADM represents a significant increase in breast cancer patients compared to control, (b) Protein expression of RAMP2 represents a statistically nonsignificant higher expression in breast cancer patients compared to control, (c) Protein expression of RAMP3 represents a significant increase in breast cancer patients compared to control and (d) Protein expression of CLR represents a statistically nonsignificant higher expression in breast cancer patients compared to control, with p-value = 0.0001, 0.2916, 0.0117 and 0.1107, respectively

Adrenomedullin and its receptors protein expression in breast cancer: Western blot analysis was conducted to detect and characterize protein expression levels. As illustrated in Fig. 3, the results indicated increased ADM, RAMP2, RAMP3 and CLR expression in breast cancer samples compared to normal. Total protein extracted from breast cancer patient samples was assessed for the presence of these proteins. The sizes of the bands visualized via SDS-PAGE autoradiography corresponded to 28 kDa for ADM (Fig. 3a), 25 kDa for RAMP2 (Fig. 3b), 35 kDa for RAMP3 (Fig. 3c), 45 kDa for CLR (Fig. 3d) and β-actin at 45 kDa, The co-expression of ADM, RAMP2, RAMP3 and CLR was confirmed, with β-actin serving as a housekeeping protein. While these results verified the presence of the proteins, the Western blot method does not provide quantitative data. Therefore, we used ELISA to quantify the protein expression levels of ADM, RAMP2, RAMP3 and CLR.

Protein expression levels of adrenomedullin and its receptors in breast cancer: Following the qualitative assessment of ADM, RAMP2, RAMP3 and CLR, protein expression levels were quantified through ELISA as shown in Fig. 4. The analysis revealed a significant increase in ADM levels (Fig. 4a), which were three times higher in breast cancer samples compared to controls (p = 0.0001; panel A). Similarly, RAMP3 expression also showed a significant difference, two times higher than in control samples (Fig. 4c) (p = 0.0117; panel C). In contrast, RAMP2 (panel B) and CLR (panel D) exhibited no significant differences in expression levels between breast cancer and control samples, with p-values of 0.2916 and 0.1107, respectively, as depicted in Fig. 4b and d, respectively.

DISCUSSION

The results reveal that ADM and its receptors play a significant role in breast cancer and could serve as independent biomarkers for early detection in this population. The study observed significantly higher expression levels of ADM, RAMP2, RAMP3 and CLR in breast cancer samples compared to controls, reinforcing the idea that their upregulation may contribute to tumor progression and metastasis in breast cancer. Understanding the molecular mechanisms behind cancer is crucial for developing effective diagnostic and treatment strategies. Early detection is particularly important for reducing cancer-related morbidity and mortality[27]. Adrenomedullin (ADM) has been identified as a potential driver of metastasis and studies indicate that elevated levels of ADM and its receptors are linked to poor outcomes in several cancers, including breast cancer[28].

Research has confirmed that elevated levels of ADM are linked to various pathways in breast cancer. The ADM levels interact with the ATP pathway, promoting programmed cell death under hypoxic conditions, which is particularly relevant given ADM's widespread expression in hypoxic tumor environments[29]. Additionally, a study found that higher ADM

gene expression correlates with lymph node metastasis, suggesting that ADM levels in plasma may serve as an independent predictor of tumor size and metastatic potential[19]. Melanoma tissues revealed high levels of ADM, CLR, RAMP2 and RAMP3, while control tissues exhibited much lower expression[30] Western blotting and ELISA results, confirmed that Saudi breast cancer patients have significantly higher levels of ADM and RAMP3 compared to controls, with RAMP2 levels slightly elevated and CLR levels remaining similar between the two groups.

The gene expression analysis revealed significant levels for all tested genes, aligning with previous studies that highlight the role of CLR, an ADM receptor, in association with accessory proteins RAMP2 or RAMP3. Another study of Dackor *et al.*[31] on genetically engineered knockout models showed that CLR is crucial for mediating AM signaling during embryonic cardiovascular development, supporting the notion that ADM interacts with both RAMP2 and RAMP3. The current study findings indicate a notably higher expression of RAMP3 compared to RAMP2 at the protein level. The RAMP3 is more active in pathological conditions, affecting migration without impacting proliferation, while RAMP2 primarily assists ADM in physiological contexts[32]. Consequently, RAMP2 levels are lower in breast cancer, suggesting that it plays a more supportive role, while RAMP3 is directly involved in cancer pathways. Although RAMP2 levels may increase in patients, they do not reach significance and CLR expression remains relatively stable since it interacts with multiple proteins.

The findings of this study highlight the potential of adrenomedullin (ADM) and its receptors (RAMP2, RAMP3 and CLR) as novel biomarkers for the early detection and diagnosis of breast cancer. The significant overexpression of ADM and its receptors in breast cancer patients suggests that these markers could be used to improve diagnostic accuracy and aid in monitoring disease progression.

Further research involving larger patient cohorts is necessary to validate these findings and assess the broader applicability of ADM as a biomarker in different populations. Additionally, clinical trials investigating ADM inhibitors or receptor blockers could help explore new therapeutic strategies for breast cancer.

The study was conducted with a relatively small sample size from a specific population (Saudi population), which limits the generalizability of the findings. Additionally, the exact mechanisms through which ADM influences tumor growth and metastasis in breast cancer remain unclear and warrant further investigation.

CONCLUSION

The ADM and its receptors are critical in breast cancer, with elevated ADM levels correlating with metastasis and potentially indicating a more aggressive tumor. This suggests a connection between ADM expression and clinical outcomes. This study supports the potential of using ADM levels as biomarkers for early breast cancer detection in the Saudi population, although larger sample sizes are necessary for validation. Additionally, targeting the AM-RAMP3 pathway may offer promising avenues for developing more effective cancer treatments or inhibitors.

SIGNIFICANCE STATEMENT

The study investigates the expression and clinical significance of adrenomedullin (ADM) and its receptors (RAMP2, RAMP3 and CLR) in breast cancer patients, its findings reveal that ADM and its receptors were significantly overexpressed in breast cancer patients compared to healthy controls, with plasma concentrations of ADM and RAMP3 showing notable increases. These findings suggest that ADM could serve as a potential biomarker for breast cancer diagnosis, offering a less invasive alternative to traditional biopsy procedures. Further research with larger cohorts is needed to confirm these results and explore the broader applicability of ADM as a diagnostic tool in breast cancer.

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REFERENCES

1. Sacco, M.A., S. Gualtieri, F. Cordasco, A.P. Tarallo and M.C. Verrina *et al.*, 2024. The role of adrenomedullin as a predictive marker of the risk of death and adverse clinical events: A review of the literature. J. Clin. Med., Vol. 13. 10.3390/jcm13164847.
2. Nakamura, M., B. Han, O. Nunobiki and K. Kakudo, 2006. Adrenomedullin: A tumor progression factor via angiogenic control. Curr. Cancer Drug Targets, 6: 635-643.
3. Benyahia, Z., C. Gaudy-Marqueste, C. Berenguer-Daizé, N. Chabane and N. Dussault *et al.*, 2022. Adrenomedullin secreted by melanoma cells promotes melanoma tumor growth through angiogenesis and lymphangiogenesis. Cancers, Vol. 14. 10.3390/cancers14235909.

4. Wang, L., M. Gala, M. Yamamoto, M.S. Pino and H. Kikuchi *et al*., 2014. Adrenomedullin is a therapeutic target in colorectal cancer. Int. J. Cancer, 134: 2041-2050.
5. Wong, L.Y.F., B.M.Y. Cheung, Y.Y. Li and F. Tang, 2005. Adrenomedullin is both proinflammatory and antiinflammatory: Its effects on gene expression and secretion of cytokines and macrophage migration inhibitory factor in NR8383 macrophage cell line. Endocrinology, 146: 1321-1327.
6. Li, H.W.R., S.B. Liao, P.C.N. Chiu, W.S.B. Yeung and E.H.Y. Ng *et al*., 2015. Effects of adrenomedullin on the expression of inflammatory cytokines and chemokines in oviducts from women with tubal ectopic pregnancy: An *in-vitro* experimental study. Reprod. Biol. Endocrinol., Vol. 13. 10.1186/s12958-015-0117-x.
7. Aiyar, N., J. Disa, M. Pullen and P. Nambi, 2001. Receptor activity modifying proteins interaction with human and porcine calcitonin receptor-like receptor (CRLR) in HEK-293 cells. Mol. Cell. Biochem., 224: 123-133.
8. Chang, C.L., Z. Cai and S.Y.T. Hsu, 2022. Sustained activation of CLR/RAMP receptors by gel-forming agonists. Int. J. Mol. Sci., Vol. 23. 10.3390/ijms232113408.
9. Wong, H.K., T.T. Cheung and B.M.Y. Cheung, 2012. Adrenomedullin and cardiovascular diseases. JRSM Cardiovasc. Dis., 1: 1-7.
10. Juaneda, C., Y. Dumont, J.G. Chabot, A. Fournier and R. Quirion, 2003. Adrenomedullin receptor binding sites in rat brain and peripheral tissues. Eur. J. Pharmacol., 474: 165-174.
11. Spoto, S., S. Basili, R. Cangemi, J.R. Yuste and F. Lucena *et al*., 2024. A focus on the pathophysiology of adrenomedullin expression: Endothelitis and organ damage in severe viral and bacterial infections. Cells, Vol. 13. 10.3390/cells13110892.
12. Gu, C., S. Park, J. Seok, H.Y. Jang, Y.J. Bang and G.I.J. Kim, 2021. Altered expression of ADM and ADM2 by hypoxia regulates migration of trophoblast and HLA-G expression. Biol. Reprod., 104: 159-169.
13. Wang, S.M. and W.L. Yang, 2009. Circulating hormone adrenomedullin and its binding protein protect neural cells from hypoxia-induced apoptosis. Biochim. Biophys. Acta Gen. Subj., 1790: 361-367.
14. Sim, R.B., J. Ferluga, H. Al-Rashidi, H. Abbow, W. Schwaeble and U. Kishore, 2015. Complement factor H in its alternative identity as adrenomedullin-binding protein 1. Mol. Immunol., 68: 45-48.
15. Jacob, A., Z. Wang, H.T. Yen and P. Wang, 2021. Human adrenomedullin and its binding protein attenuate tissue injury and inflammation following hepatic ischemia reperfusion in rabbits. Heliyon, Vol. 7. 10.1016/j.heliyon.2021.e07845.
16. Shindo, T., M. Tanaka, A. Kamiyoshi, Y. Ichikawa-Shindo, H. Kawate and T. Sakurai, 2022. Receptor activity modifying protein RAMP sub-isoforms and their functional differentiation, which regulates functional diversity of adrenomedullin. Biology, Vol. 11. 10.3390/biology11050788.
17. Liang, Y.L., M.J. Belousoff, M.M. Fletcher, X. Zhang and M. Khoshouei *et al*., 2020. Structure and dynamics of adrenomedullin receptors AM_1 and AM_2 reveal key mechanisms in the control of receptor phenotype by receptor activity-modifying proteins. ACS Pharmacol. Transl. Sci., 3: 263-284.
18. Russo, A.F. and D.L. Hay, 2023. CGRP physiology, pharmacology, and therapeutic targets: Migraine and beyond. Physiol. Rev., 103: 1565-1644.
19. Oehler, M.K., D.C. Fischer, M. Orlowska-Volk, F. Herrle, D.G. Kieback, M.C.P. Rees and R. Bicknell, 2003. Tissue and plasma expression of the angiogenic peptide adrenomedullin in breast cancer. Br. J. Cancer, 89: 1927-1933.
20. Liu, L.L., S.L. Chen, Y.H. Huang, X. Yang and C.H. Wang *et al*., 2020. Adrenomedullin inhibits tumor metastasis and is associated with good prognosis in triple-negative breast cancer patients. Am. J. Transl. Res., 12: 773-786.
21. Kaur, B., F.W. Khwaja, E.A. Severson, S.L. Matheny, D.J. Brat and E.G. van Meir, 2005. Hypoxia and the hypoxia-inducible-factor pathway in glioma growth andangiogenesis. Neuro-Oncology, 7: 134-153.
22. Qannita, R.A., A.I. Alalami, A.A. Harb, S.M. Aleidi and J. Taneera *et al*., 2024. Targeting Hypoxia-Inducible Factor-1 (HIF-1) in cancer: Emerging therapeutic strategies and pathway regulation. Pharmaceuticals, Vol. 17. 10.3390/ph17020195.
23. Rizwan, A., C. Bulte, A. Kalaichelvan, M. Cheng and B. Krishnamachary *et al*., 2015. Metastatic breast cancer cells in lymph nodes increase nodal collagen density. Sci. Rep., Vol. 5. 10.1038/srep10002.
24. Place, A.E., S.J. Huh and K. Polyak, 2011. The microenvironment in breast cancer progression: Biology and implications for treatment. Breast Cancer Res., Vol. 13. 10.1186/bcr2912.
25. Ji, R.C., 2017. Lymph nodes and cancer metastasis: New perspectives on the role of intranodal lymphatic sinuses. Int. J. Mol. Sci., Vol. 18. 10.3390/ijms18010051.
26. Alwarthan, H.A.M., M.S. Al Anazi, N.R. Parine, R.S. Bhat, G. Alamro, F. Aljarbou and S.K. Al-Daihan, 2019. The expressional level of tankyrase-1 gene and its regulation in colorectal cancer in a Saudi population. BIOCELL, 43: 51-58.
27. Gulati, P. and C.V. Singh, 2024. The crucial role of molecular biology in cancer therapy: A comprehensive review. Cureus, Vol. 16. 10.7759/cureus.52246.

28. Paré, M., C.Y. Darini, X. Yao, B. Chignon-Sicard and S. Rekima *et al*., 2020. Breast cancer mammospheres secrete Adrenomedullin to induce lipolysis and browning of adjacent adipocytes. B MC Cancer, Vol. 20. 10.1186/s12885-020-07273-7.
29. Yang, H., Y.H. Geng, P. Wang, H.Q. Zhang, W.G. Fang and X.X. Tian, 2022. Extracellular ATP promotes breast cancer chemoresistance via HIF-1α signaling. Cell Death Dis., Vol. 13. 10.1038/s41419-022-04647-6.
30. Chen, P., Y. Huang, R. Bong, Y. Ding and N. Song *et al*., 2011. Tumor-associated macrophages promote angiogenesis and melanoma growth via adrenomedullin in a paracrine and autocrine manner. Clin. Cancer Res., 17: 7230-7239.
31. Dackor, R.T., K. Fritz-Six, W.P. Dunworth, C.L. Gibbons, O. Smithies and K.M. Caron, 2006. Hydrops fetalis, cardiovascular defects, and embryonic lethality in mice lacking the *Calcitonin receptor-like receptor* gene. Mol. Cell. Biol., 26: 2511-2518.
32. Ichikawa-Shindo, Y., T. Sakurai, A. Kamiyoshi, H. Kawate and N. Iinuma *et al*., 2008. The GPCR modulator protein RAMP2 is essential for angiogenesis and vascular integrity. J. Clin. Invest., 118: 29-39.